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Influence of lactobacilli on the adhesion of *Staphylococcus aureus* and *Candida albicans* to fibers and epithelial cells

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The ability of organisms to adhere to and form biofilms on fibrous materials is believed to be an important initiating step in the induction of several diseases, such as toxic shock syndrome. Using an *in vitro* assay, a moderately hydrophobic strain of *Staphylococcus aureus* (water contact angle 35°) and a hydrophilic *Candida albicans* (shown by a hexadecane test) were highly adherent to commercial diaper fibers. The lumen side of the diaper was porous and the fibers were very hydrophobic (>140°), but the internal section was very hydrophilic (0°), presumably for adsorption purposes. There was evidence that adhesion of the pathogens was inhibited when one of five *Lactobacillus* strains was present. Surfaces precoated with lactobacilli inhibited staphylococcal adhesion by 26–97%, and candida by 0–67%. When the lactobacilli were used to challenge adherent pathogens, there was 99% displacement of the *S. aureus* and up to 91% displacement of *C. albicans*. Hydrophobic *L. acidophilus* 76 (54°) and T-13 (80°) were the most effective of five *Lactobacillus* isolates tested at interference by precoating. The moderately hydrophilic *L. casei* var *rhamnosus* GR-1 (33°) was the most effective at displacing the yeast. Experiments with uroepithelial cells also showed that the lactobacilli could significantly interfere with the adhesion of both pathogens to the cells. The results demonstrate the rapidity with which two pathogens adhered to fibers and epithelial cells, and raised the possibility that members of the normal female urogenital flora might interfere with infections caused by these organisms.

Keywords: pathogens; adhesion; interference; fibers

The association between tampon use and toxic shock syndrome (TSS) has been well documented, if not fully understood. The correlation between the use of highly absorbant polyacrylate-containing tampons and disease [2,30] indicates that biomaterials can play an important role in the process. The absorbance of *Staphylococcus aureus* TSS toxin-1 (TSST-1) to various materials [31], further illustrates the importance of contact with the substratum.

Fibrous materials, other than tampons, are placed against the urogenital mucosa, for example, incontinence pads which are used to drain and collect urine. In 1992, over 1.6 billion incontinence pads were sold in North America [22]. The alternative to pads is catheter usage, and infections have been reported to arise in almost all patients who use this management technique, while the infection rate is 40% for pad users [16]. The reasons why infections arise with the use of pads, and the possible involvement of the pad material is not understood.

Little is known about the role of materials in infections associated with diapers used on babies. In 1992, over 56 billion diapers were sold in North America, with rates of infected skin rash conservatively estimated at 10% of babies; this amounts to a large patient population. Admittedly, factors such as friction, irritation, skin wetness, changes in skin pH, exposure to fecal components and enzymes can induce inflammation in the diaper region. When infections do occur, they are mainly due to *Candida albicans* and *S. aureus* [1,4,20]. The adhesion of organisms to diaper material seems feasible, as does the hypothesis that such adhesion might play a role in pathogenesis. One study of forty patients with diaper dermatitis showed *C. albicans* from the stool to be the causative organism in 37 (93%) of cases [7]. The ability of fungi to bind to tissues and foreign bodies has been associated with pathogenesis [33], but the actual diaper material does not apparently contribute to microbial growth [12]. However, it does appear that the large surface area provides a means whereby organisms can come into contact with the skin surface for periods of up to and over 8 h. *Candida albicans* can, of course, cause urinary tract infections in association with foreign bodies [17], but these organisms are renowned for causation of vaginal candidiasis [19].

Etiological studies have shown an association between a recent history of vaginitis and TSS [15], perhaps suggesting a correlation with disruption of the normal microbial ecology. Studies from our laboratory have indicated that lactobacilli can interfere with the adhesion of pathogens to surfaces [8,25,28]. In addition, a recent clinical trial showed a three-fold decrease in candida vaginitis for patients who consumed 8 ounces of yogurt containing *Lactobacillus acidophilus*, although the mechanism of protection and role of lactobacilli were not determined [10]. The industrial potential to apply specifically designed lactobacillus technology *in vivo* is large [21] and studies have shown some degree of success [5,23,24] without any outbreaks of fungal super-infections.

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The aims of the present study were to determine if microorganisms can adhere to commercially available fibers, and if lactobacillus strains can interfere with their binding.

Materials and methods

Microorganisms

A *S. aureus* and a *C. albicans* strain were isolated from patients with urinary tract infections. They were stored at −70° C and cultured for 18 h at 37° C in brain heart infusion yeast extract broth and MYGP broth (malt extract, 3 g; yeast extract, 3 g; bacteriological peptone, 5 g; glucose, 10 g; distilled water, 1 L, pH 5.5) prior to assay. Five lactobacillus isolates were selected for their different hydrophobicity, as described previously [26]. These included three human strains, *L. casei* 36, *L. casei* var *rhamnosus* GR-1 and *L. acidophilus* 76, plus two poultry isolates *L. acidophilus* T-13 and *L. fermentum* B-54. Strains B-54 and GR-1 had been found to be very adherent to uroepithelial cells and had been used in studies to prevent urinary tract infections in adult females. The organisms were grown at 37° C for 24 h in MRS broth.

Contact angle measurements

Contact angle measurements were measured as described previously [26,32]. Briefly, bacterial lawns were prepared on cellulose acetate membrane filters (Millipore, Nepean, Ontario, 0.45-μm pore size) by negative pressure. Filters were glued to a thin layer of dental wax on an aluminum disc. The organisms were dried at 37° C for 2–3 h. Contact angles were measured with two droplets of water per filter. In the case of the diaper material, the lumenal layer was measured separately from the inner layers.

Adhesion to diapers

The adhesion experiments have been described [28]. In summary, 2 cm^2 sections of diaper (Huggies, Kimberly Clarke, Neenah, WI, USA) were placed in a dish and microbial suspensions of 10^8 organisms per ml phosphate buffered saline (PBS, pH 7.1) were added for 1 h at 37° C. The time frame was selected as the aim was to examine initial adhesion rather than stages of biofilm formation which occurred after several hours, and the 1-h protocol had been used effectively in previous experiments. Also, lactobacillus viability dropped off dramatically in PBS after several hours. Three sets of experiments were carried out: (i) equal amounts of *S. aureus*, *C. albicans* and one of a lactobacillus strain were added and incubated for 1 h; (ii) in precoating experiments, the lactobacilli were incubated with the diaper section for 1 h prior to 1-h challenge with each pathogen; (iii) in displacement experiments, each pathogen was incubated with the material for 1 h then lactobacilli were added and incubated for another hour. After incubation, the material was rinsed in PBS to remove unattached bacteria, placed in 5 ml PBS, sonicated to detach adherent organisms, and then dilution plated onto MRS, mannitol salt and MYGP agar.

The results were presented as the number of viable adherent organisms isolated. Scanning electron microscopy [27] verified the structural nature of the fibrous material (Figure 1), and the extent of bacterial adhesion. It also allowed verification that the sonication technique had been effective at removing the adherent organisms. The experiments were performed in triplicate.

In addition, an electron microscopy examination was undertaken to study whether *S. aureus* DL1 expressing TSST-1 could adhere to tampon fibers (Tampax, Tambrands Canada Inc., Toronto). Also, the ability of a TSST-1 strain of *S. aureus* DL1 to adhere to diaper material was tested.

Adhesion to cells

As the diapers come into contact with skin and as microbial adhesion on the epithelium is likely also important in pathogenesis, the ability of lactobacilli to displace *S. aureus* and *C. albicans* and to prevent their adhesion was tested using uroepithelial cells recovered from the sediment of fresh mid-stream urine [25]. These experiments were also carried out in triplicate at 37° C over 1 h, with the numbers of adherent bacteria being quantified by light microscopy.

Results

The diaper material was porous and comprised many strands of fibrous material in separate layers, as shown in Figure 1. The layer which would be closest to the skin was extremely hydrophobic with a water contact angle of >140°. However, the diaper appeared to contain an inner layer, presumably for absorption, which was hydrophilic, with a contact angle of 0°. *S. aureus* was relatively hydrophobic (35°), and the lactobacilli ranged in their surface properties as shown in Table 1. The morphology of the yeast cells made it impossible to obtain with accuracy a water contact angle measurement. Thus, the organisms were tested for their hydrophobicity using the microbial adhesion to hexadecane test (MATH) described by Rosenberg [29]. This showed the strain to be very hydrophilic.

The seven organisms all adhered well to the fibers, especially *S. aureus*, as shown in Table 1 and illustrated in Figure 2, which shows the presence of glycocalyx material

Figure 1 Scanning electron micrograph showing the strand-like nature of the diaper material. Bar = 87.7 μm

Table 1 The water contact angle and adhesion of microorganisms to 2 cm^2 sections of fibrous diaper material

Organisms	Water contact angle (°)	Adhesion[a]
C. albicans	0[b]	$3.2 \pm 2.3 \times 10^3$
S. aureus	35	$6.8 \pm 1.2 \times 10^5$
L. casei 36	19	$6.9 \pm 2.4 \times 10^3$
L. casei var *rhamnosus* GR-1	33	$1.4 \pm 0.2 \times 10^7$
L. acidophilus 76	54	$1.9 \pm 0.4 \times 10^3$
L. acidophilus T-13	80	$4.2 \pm 0.5 \times 10^3$
L. fermentum B-54	105	$1.4 \pm 0.8 \times 10^4$

[a] Expressed as number of viable organisms recovered per 2 cm^2 section of diaper ± standard deviation
[b] Determined to be hydrophilic by the MATH test [26]

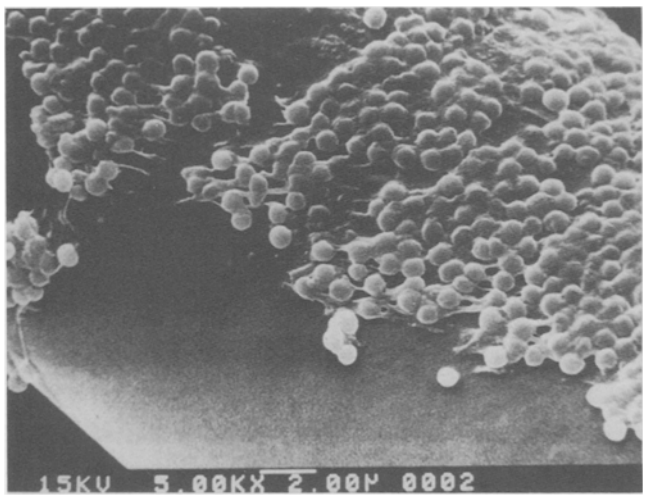

Figure 2 Scanning electron micrograph showing *S. aureus* adherent to a fiber of a diaper within 1 h of incubation. The glycocalyx, slime material is evident linking the organisms onto the surface. Bar = 2 μm

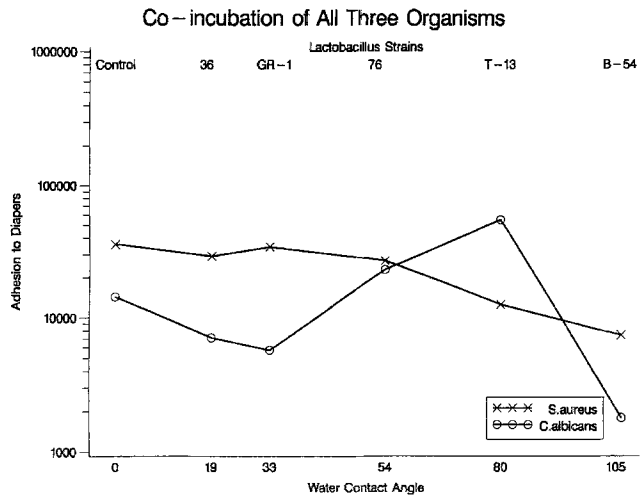

Figure 3 Viable adhesion data for experiments whereby both pathogens and one of five *Lactobacillus* strains of differing hydrophobicity were co-incubated with diaper material

linking the cells in initial biofilm mode and binding the bacteria to the fiber. The highly hydrophobic *L. fermentum* B-54 adhered best of the normal flora.

Effect of coincubation: When the two pathogens were coincubated, there was a 13-fold increase in the number of *C. albicans* adherent but a 20-fold decrease in the number of *S. aureus* adherent. Coincubation of the two pathogens with one of five lactobacilli reduced the adhesion of *S. aureus* by 10% (GR-1), 29% (76), 76% (T-13: $P = 0.0086$) and 80% (B-54: $P < 0.0001$), and reduced the *C. albicans* adhesion by 45% (36: $P = 0.0003$), 55% (GR-1: $P = 0.0003$) and 86% (B-54: $P < 0.0001$) (Figure 3). The correlation coefficients were −0.81 and −0.38 for *S. aureus* and *C. albicans* respectively in relation to the hydrophobicity of lactobacilli.

Pretreatment with lactobacilli: Pretreating the diapers with lactobacilli resulted in a significant reduction in adherent *S. aureus* by 26% (B-54: $P = 0.0967$), 57% (GR-1: $P = 0.0031$), 62% (T-13: $P = 0.0049$), 85% (36: $P = 0.0006$) and 97% (76: $P < 0.0001$) (Figure 4). Adhesion of *C. albicans* was significantly reduced by pretreatment by *L. acidophilus* 76 (60%: $P = 0.0002$) and T-13 (67%: $P = 0.0006$). There was no correlation between

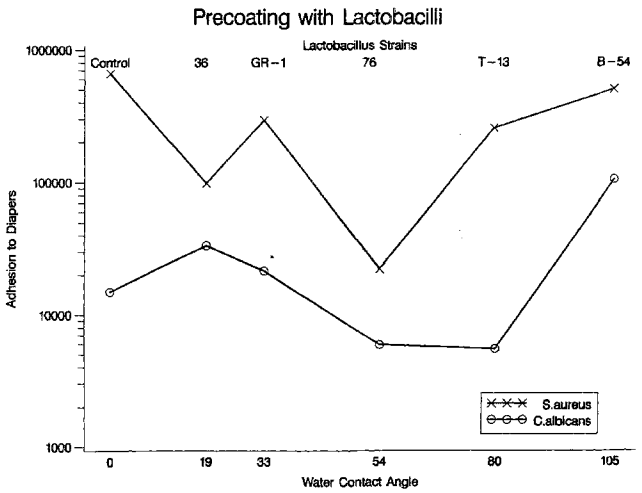

Figure 4 Viable adhesion data for experiments whereby one of five *Lactobacillus* strains was incubated with diaper material followed by challenge with one of two pathogens

reduction in adhesion and hydrophobicity of the lactobacilli (0.07).

The pathogens, especially *S. aureus*, were highly adherent to uroepithelial cells (Table 2). Pretreatment of

Table 2 Adhesion of *S. aureus* and *C. albicans* to adult female uroepithelial cells with and without pretreatment using lactobacilli, and after being challenged by lactobacilli

Treatment	*C. albicans* per cell	*S. aureus* per cell
Control	32	118
Pretreatment with:	vs Control[a]	vs Control
L. casei 36	19 $P = 0.043$	69 $P = 0.008$
L. casei var *rhamnosus* GR-1	25 $P = 0.068$	85 $P = 0.008$
L. acidophilus 76	17 $P = 0.014$	71 $P = 0.008$
L. acidophilus T-13	20 $P = 0.048$	75 $P = 0.008$
L. fermentum B-54	22 $P = 0.027$	76 $P = 0.016$
Displacement		
L. casei 36	15 $P = 0.020$	58 $P = 0.004$
L. casei var *rhamnosus* GR-1	19 $P = 0.032$	73 $P = 0.010$
L. acidophilus 76	14 $P = 0.013$	61 $P = 0.007$
L. acidophilus T-13	15 $P = 0.017$	56 $P = 0.001$
L. fermentum B-54	20 $P = 0.015$	69 $P = 0.005$

[a] Statistical analysis of $n = 5$ duplicates by paired t-test

the cells with lactobacilli resulted in a significant (28–42%) reduction in adhesion by staphylococci, and also against *C. albicans* (22–46%), as verified by a paired t-test analysis.

Displacement of pathogens: The use of lactobacilli to challenge the fibers which had been pre-incubated with staphylococci, resulted in a 99% removal of the pathogens with P values <0.0001 for strains *L. casei* 36, *L. casei* var *rhamnosus* GR-1 and *L. fermentum* B-54, 0.0002 for strain *L. acidophilus* 76 and 0.0006 for *L. acidophilus* T-13 (Figure 5). The correlation coefficient was −0.69. The displacement effect was also significant against the yeast using *L. acidophilus* T-13 (68%: $P = 0.0224$), *L. casei* 36 (77%: $P = 0.0031$), *L. acidophilus* 76 (86%: $P = 0.0012$) and *L. casei* var *rhamnosus* GR-1 (91%: $P = 0.0001$) and the correlation coefficient was 0.32.

There was also significant displacement of *C. albicans* (38–56%) and *S. aureus* (38–53%) from epithelial cells by lactobacilli (Table 2).

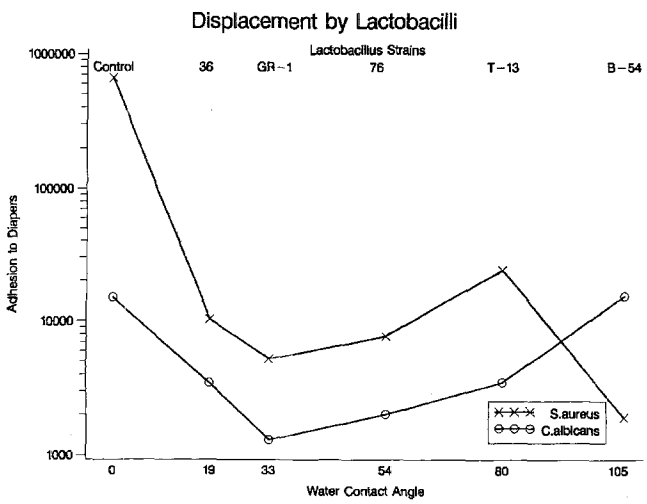

Figure 5 Viable adhesion data for experiments whereby one of five *Lactobacillus* strains was used to challenge a material which had been exposed to one of two pathogens

Additional experiments showed that toxic shock syndrome-isolate *S. aureus* DL1, expressing TSST-1, was highly adherent to the diaper material (mean = 1.3 ± 0.07 × 10^5 organisms per diaper section). An electron microscopy examination showed that tampon fibers (Tampax, Tambrands Canada Inc, Toronto), while not exactly the same as the diaper material in appearance used here, were of a similar fibrous, strand-like nature to which the *S. aureus* DL1 strain adhered well (Figure 6).

Discussion

The results show that a moderately hydrophobic clinical isolate of *S. aureus* and a hydrophilic isolate of *C. albicans* were capable of adhering in high numbers to fibrous diaper materials. In particular, the staphylococci showed signs of biofilm formation even within 1 h of binding.

In the presence of *S. aureus* the numbers of adherent *C. albicans* increased significantly. Similar findings of this nature have been reported for adherence of *Candida* to acrylic in the presence of *S. mutans* [3, 34]. Addition of lactobacilli in the present study caused a marked effect on adhesion, with significant reductions in many cases, and most consistently in displacement studies. It has been suggested that disruption of the normal indigenous microflora, especially lactobacilli, could predispose women to toxic shock syndrome [6]. The implication from the present study, is that supplementation of the microflora with selected lactobacilli could perhaps reduce the risk of infection.

Four strains of lactobacilli (B-54, T-13, 76 and 36) interfered with adhesion of *E. coli* and *E. faecalis* to catheters [28], and hydrophilic *L. casei* 36 has been found able to displace enterococci from glass and fluorinated ethylene propylene under flow conditions [18]. The latter study raised the possibility that lactobacillus metabolic by-products could influence displacement of organisms from surfaces. Certain lactobacilli strains, including GR-1, B-54, T-

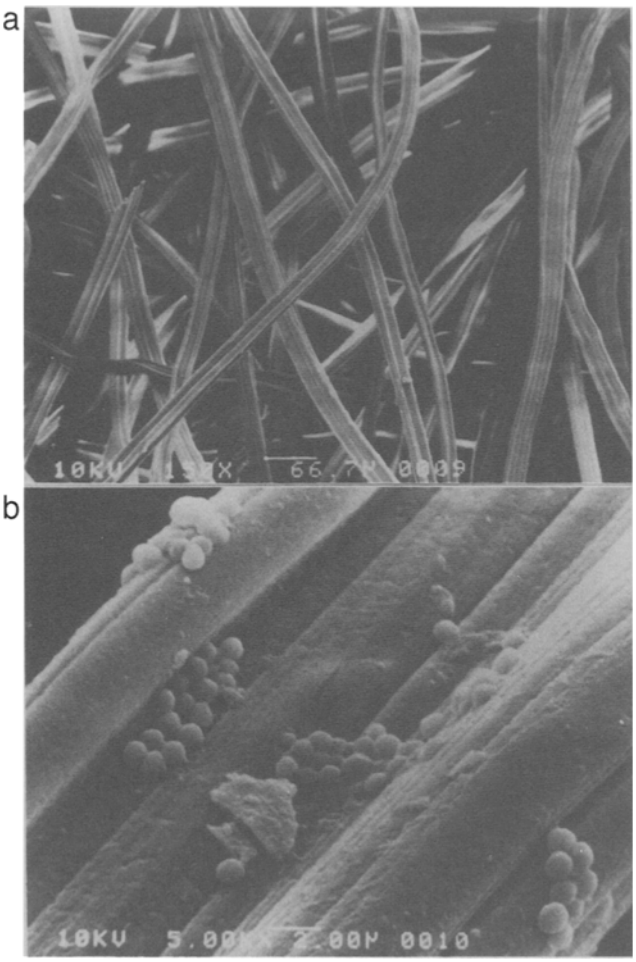

Figure 6 Scanning electron micrograph of (**a**) Tampax tampon fibers showing the strand-like nature of the material (Bar = 66.7 μm), and (**b**) *S. aureus* DL1 expressing TSST-1 adherent to the surface after 1 h incubation. Bar = 2 μm

13 and 76, express properties inhibitory to the growth and attachment of various organisms, including *S. aureus* [25], however, specific biosurfactants involved in displacement have not yet been isolated and identified.

The interference effect of lactobacilli was not related to their hydrophobicity, and it appears that other attributes play more of a role in the interference process. This is in agreement with a recent study which showed that adhesion of lactobacilli to diaper material was not related to hydrophobicity *per se* [27]. The ability to interfere with adhesion of uropathogenic *C. albicans* and *S. aureus* differed depending upon which organism had first access to the surface. When all the organisms were present at the same time, the presence of highly hydrophobic *L. fermentum* B-54 interfered the most with adhesion of staphylococci and candida. However, moderately hydrophobic *L. acidophilus* 76, once adherent to the surface, was the most effective at preventing attachment of pathogens. In displacement studies, *L. casei* var *rhamnosus* GR-1 was the most effective. On two occasions, *L. acidophilus* T-13 coincubated with the pathogens and *L. fermentum* B-54 precoating the diaper and being challenged with *C. albicans*, there was increased adhesion of the yeast compared to controls. There is no apparent thermodynamic or coaggregation explanation for this, and it is unclear as to why it occurred. It is possible that the underlying hydrophilic layer affected the adhesion profiles, but to test this theory would require delicate separation of the layers and subsequent exposure to organisms. It does seem feasible that organisms might 'see' the underlying layer and adhere to it. If this occurred, it could perhaps affect pathogenesis by providing a substrate for adhesion and toxin production. Further studies would be needed to test these hypotheses.

Adhesion of *C. albicans* to plastic surfaces is influenced more by environmental circumstances than hydrophobicity of the organism's surface [11]. The actual adhesion of *C. albicans* found here was high, as in other experiments where extracellular matrix proteins, such as type IV collagen, fibronectin and laminin were present [13].

The experiments with epithelial cells also provided convincing *in vitro* evidence that all five lactobacilli interfered with the binding of highly adherent staphylococci and candida, by way of precoating and displacement. It has been suggested that hydrophilic *C. albicans* are not highly adherent to cells, and therefore not as virulent as adhesive hydrophobic strains [9,14]. Although no hydrophobic candida were tested here, the adhesion levels to cells were high and also to biomaterials for the isolate used.

Correlation with the clinical situation was not the purpose of this study and no definitive conclusions can be drawn. However, two uropathogens and one *S. aureus* DL1 which expressed TSST-1, were highly adherent to fibrous material with the ability to form biofilms rapidly. Even although it has not been proven that incontinence pads or diapers are a vehicle for pathogenesis, they could still act as a delivery system for therapies, such as lactobacilli, to prevent and perhaps treat infections. The fact that *L. fermentum* B-54 and *L. casei* var *rhamnosus* GR-1 showed some effect, and that they have already been used in humans to alleviate recurrent bladder infections [5,24], suggests that the technology is worthy of industrial interest. Confirmation that lactobacilli could reduce the risk of infections related to incontinence pads, diapers and tampons would constitute a very significant breakthrough and might prove a preferred substitute to some agents currently used to manage these urogenital and skin diseases.

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